Subgroup: Membrane Structure & Assembly

1-Subg
New Tools For Studies Of Membrane Protein Dimerization In Mammalian Membranes
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Receptor Tyrosine Kinases (RTKs) play key roles in cell growth, differentiation, metabolism, and migration. These single-pass receptors conduct biochemical signals by dimerizing in the plasma membrane. The process of lateral dimerization, which controls the distribution between inactive monomers and active dimers, serves as a key regulator of the biochemical processes that determine cell fate. Enhanced dimerization leads to persistent autocrine activation and tumorigenesis, or impaired growth. An understanding of the dimerization process as a function of interaction energies, protein concentration and ligand concentration, is lacking. Our laboratory is developing methodologies that yield quantitative information about RTK dimerization and activation in cellular membranes. These methods will enable biomedical researchers to study the quantitative aspects of signal transduction in the context of the biological membrane.

2-Subg
Small, Dynamic Domains in Lipid Membranes near a Miscibility Critical Point
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We study giant lipid vesicles as a model of plasma membranes in cells. We find that liquid domains appear on the surface of vesicles containing at minimum a high melting temperature lipid, a low melting temperature lipid, and cholesterol. These three components separate into two phases. Near a miscibility critical point, the edges of the domains fluctuate, which indicates a low line tension at the boundary between the domain and the surrounding membrane. At higher temperatures, above the critical point, domains are replaced by submicron fluctuations. We find that the size of the largest fluctuations (the correlation length) and their compositions (the order parameter) scale in a way that is consistent with the universality class of the two-dimensional Ising model. This knowledge has a direct application: we can predict at what temperature our membranes should contain domains of any particular size, even at length scales below our optical resolution.
REFERENCES:
1. S.L. Veatch, O. Soubias, S.L. Keller, and K. Gawrisch, Critical Fluctuations in Domain-Forming Lipid Mixtures, PNAS, 104, 17650-17655, 2007.
2. A.R. Honerkamp-Smith, P. Cicuta, M.D. Collins, S.L. Veatch, M. den Nijs, M. Schick, and S.L. Keller, Line Tensions, Correlation Lengths, and Critical Exponents in Lipid Membranes near Critical Points, Biophys. J., 95, 236-246, 2008.
3. A.R. Honerkamp-Smith, S.L. Veatch, and S.L. Keller, An Introduction to Critical Points for Biophysicists: Observations in Lipid Membranes, Biochim. Biophys. Acta, in press, 2008.

3-Subg
Fascinating Vesicles
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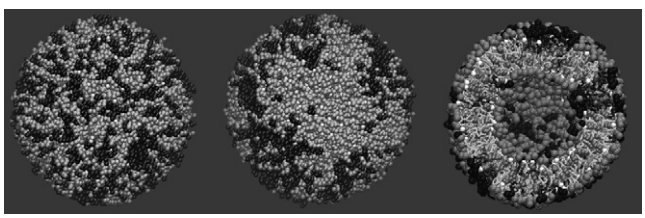
Lipid vesicles, or liposomes, are widely used in biophysical studies as mimics of either complete cells or cell parts, and have a potential wide range of biotechnological applications. Here I will present the latest results of our group on simulations of vesicles, based on the coarse grained MARTINI forcefield[1]. I will discuss equilibration issues of vesicles[2], and show the formation of raft-like domains in ternary systems composed of saturated and unsaturated lipids together with cholesterol[3]. The effect of osmotic pressure on the structure and stability of lipid vesicles and the response of membrane-embedded mechano-sensitive protein channels will also be discussed[4].



Domain formation in a three component vesicle. Starting from a randomized mixture (left), a saturated-PC/cholesterol enriched liquid-ordered domain is formed on a microsecond time scale (middle and rightmost image). Light/dark grey denotes saturated/poly-unsaturated lipids. Cholesterol is depicted with a white hydroxyl group. Water not shown.

References
1. S.J. Marrink, H.J. Risselada, S. Yefimov, D.P. Tieleman, A.H. de Vries, *J. Phys. Chem. B*, 2007, **111**, 7812.
2. H.J. Risselada, A.E. Mark, S.J. Marrink, *J. Phys. Chem. B*, 2008, **112**, 7438.
3. H.J. Risselada, S.J. Marrink, *Proc. Nat. Acd. Sci. USA*, 2008, in press.

4-Subg
Thermodynamics of Membrane Partitioning and Self-association of Transmembrane Helices: Impact of Lipid Composition
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Membrane partitioning and self-association of transmembrane helices (TMHs) have been extensively investigated using model TMHs to elucidate the driving forces of membrane protein folding. We designed the inert hydrophobic model TMH $(AALALAA)_3$ to obtain thermodynamic parameters related to the steps. The formation of the antiparallel dimer was detected by fluorescence resonance energy transfer between fluorescent labeled peptides [1]. Stronger dimerization was observed in thicker membranes and at lower temperatures ($\Delta G = -9 - -26$ kJ mol^{-1}), driven by large negative ΔH values ($-18 - -80$ kJ mol^{-1}). The enthalpy changes for helix-helix interaction can be well explained by electrostatic interaction between helix macrodipoles in different dielectric environments. The incorporation of cholesterol and PE also facilitated the dimerization by large negative ΔH values.
The partitioning process was also investigated based on the transfer of the helix between vesicles [2]. Under hydrophobic mismatch conditions up to ~7 Å, the helix partitioning became unfavorable up to $+7$ kJ mol^{-1}, hampered by an increase in entropic (up to $+20$ kJ mol^{-1}) and enthalpic (up to $+66$ kJ mol^{-1}) terms in thinner and thicker membranes, respectively. The obtained thermodynamic parameters were reasonably explained assuming that hydrophobic mismatch induces aqueous exposure or membrane burial of the helix termini, respectively. I also present a design of a water-soluble TMH for the direct measurement of the partitioning process. Furthermore, I will introduce a novel method for quick fluorescent labeling of membrane proteins to detect TMH interactions in living cell membranes [3].
[1] Y. Yano and K. Matsuzaki, *Biochemistry* 45, 3370 (2006).
[2] Y. Yano et al., *Biochemistry* 45, 3379 (2006).
[3] Y. Yano et al., *ACS Chem. Biol.* 6, 341 (2008).

5-Subg
Structure And Bending Rigidity Of Fully Hydrated Lipid Bilayers With Added Peptides And Cholesterol Using Diffuse X-ray Scattering
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Our lab uses x-ray synchrotron radiation to study fully hydrated stacks of ~2000 lipid bilayers. Like cell membranes, these oriented bilayers fluctuate when they have their full complement of water. These fluctuations produce diffuse x-ray scattering which allows us to determine both structure and the rigidity (bending modulus K_C). We have more recently been adding two classes of peptides, channel-forming and fusion, to these lipid bialyers. By comparing our experimental form factor data to form factors obtained from MD simulations by Peter Tieleman we confirm that the channel-former, alamethicin, inserts into DOPC membranes in a transmembrane fashion. We also find that alamethicin thins DOPC by 3 Å and diC22:1PC membranes by 4 Å at 1:10 P/L mole ratio. Two peptides from the gp41 protein on the ectodomain of the HIV-1 virus, fusion peptide FP23 at the N-terminus, and the cholesterol-sequestering CRAC motif peptide near the transmembrane region, were also added to lipids of varying thickness and chain unsaturation (BJ (2007) 93:2048, BBA (2008) 1778:1120). The CRAC-motif LWYIK peptide thinned SOPC membranes by 3 Å at 1:9 P/L ratio. All peptides caused a softening of the membranes, but the decrease in the bending modulus, K_C, was greatest for the FP23 peptide, which we have suggested is related to its special ability to promote highly curved fusion intermediates at the HIV/T-cell fusion site. We have also added cholesterol to bilayers and have obtained the remarkable result that cholesterol does not stiffen unsaturated DOPC bilayers, in striking contrast to the well known result that cholesterol greatly stiffens saturated DMPC bilayers (Phys. Rev. Letts. (2008) 100:198103).

6-Subg
Control of Hydrophobic Helix Topography in Membranes by Lipid Composition
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The sequence of hydrophobic helices can modulate topography in terms of both the stability of the transmembrane (TM) configuration (relative to a *membrane-bound* non-TM state) and the transverse position of the helix in